CHAPTER 3

RESULTS AND DISCUSSION

In the course of studies on the chemical contents of the stem bark of the plant *Pseuduvaria rugosa*, the author reported the isolation of seven different aporphine alkaloids together with two other different classes of compounds. Four of them belong to the rare 4,5-dioxoaporphine whereas the remaining possess the typical oxoaporphine skeleton. Only one of them is a new compound while others have been separated previously from other plant especially from other *Pseuduvaria* species. In addition, two terpenoids have been isolated from petroleum-ether extract fraction and both of them are new compounds. Their spectrums have been analyzed thoroughly supported by 2D-NMR.

3.1 Alkaloid Extract of *Pseuduvaria rugosa*

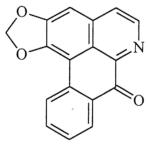
About 1.5 kg of finely ground dried stem barks of the plant was defatted with petroleum-ether (60-80°C) and then was extracted with dichloromethane using Soxhlet extractor. The petroleum-ether extracts were evaporated to dryness and consequently yield a gummy brown liquid. Several non-alkaloidal compounds mostly terpenes have been detected but only two compounds can be fully isolated and their structure have been determined by spectral analysis and are labeled as PR8 and PR9.

Aside from that, by using the conventional methods of alkaloid extraction as described in the experimental section, yielded 2.4 % crude bases. This

dichloromethane extract was then introduced to column chromatography over silica gel with dichloromethane and methanol as eluating solvents. A total of fractions were collected and grouped into series of fractions after monitoring with TLC. Further column chromatography with suitable solvents, preparative thin layer chromatography as well as High-Performance-Liquid-chromatography (HPLC) technique has also been used to isolate and subsequently purify the individual compound.

Several yellow compounds labeled as PR4, PR5, PR1 and PR2 have been eluted. However, PR5 was further purified using HPLC techniques. Subsequently, PR3 was separated as an orange compound followed by PR6 as red compound. TLC of some of the alkaloid fraction also showed traces of purple, blue, red and other colorful compounds. Unfortunately, the proportions of these were too small to allow isolation and positive identification.

3.1.1 Structural Elucidation of Compound Labeled PR1



This compoud was crystallized from chloroform as yellow needles, which melted with decomposition at 270°-272°C. An oxoaporphinic nature was based on its intense yellow color and deep red coloration it produced in acid medium.

The presence of a highly unsaturated chromophoric system of an oxoaporphine was further supported by the UV spectrum with absorption bands at 247.5, 269 and 302 nm. Moreover, the spectrum was shifted to longer wavelengths, giving a series of undulating maxima at 256.5, 280 and 334 nm on acidification in 0.1 N HCl.

The existence of a highly conjugated ketone function was evidenced from the significant peak at 1664 cm^{-1} in its IR spectrum. IR is an extraordinarily powerful tool for diagnosing the nature and the chemical environment of a carbonyl group in molecule of unknown structure. Therefore, the carbonyl group of this compound, which are conjugated with a double bond or aromatic ring are lowered by 30 cm^{-1} compared to the unsaturated aliphatic ketones that absorb near 1715 cm^{-1}. The absorption peak attributable to a methylenedioxy group was

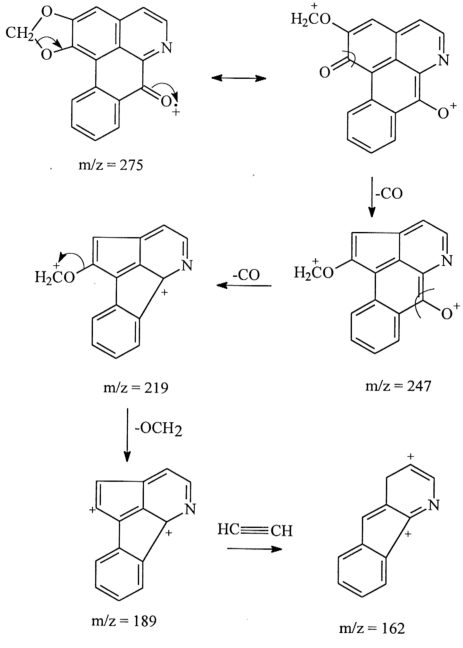
observed at 1244 cm^{-1}. In addition, the bands between 3017 cm^{-1} and 2928 cm^{-1} were due to the C-H stretching of the aromatic rings and the alkyl groups. A strong peak observable at 750 cm^{-1} was due to the C-H out-of- plane deformation of four adjacent aromatic protons in ring D. However, a medium band at 967.5 cm^{-1} was present as a result of the C-H out-of-plane deformation of a single isolated aromatic proton, which is attributable to H-3[53].

This compound has a molecular formula $C_{17}H_9NO_3$ as established from LC-MS in accordance with the appearance of very significant peak at $[M+1]^+$ 276. Therefore, the molecular ion peak is at m/z 275 while other significant fragmentations of compound A like m/z 247, 219, 189,188 and 162 are illustrated in Scheme 10. Fragmentation peaks at m/e 247 (M-CO) and 219 (M-CHO) are due to the cleavage of the ketone group. Subsequently, a methylenedioxy group (M^+-CH_2O) was lost to give the base peak at m/z 189. Then, the compound underwent rearrangement to eliminate HC≡CH unit giving rise to a peak at m/z 162.

The ^{1}H-NMR spectrum revealed the characteristic AB quartet typical of H-4 and H-5 at δ 8.9 and δ 9.2 respectively with a coupling constant of 5.3 Hz. However, the proton that is adjacent to the N atom is slightly deshielded and as a result, resonates at lower field compared to the former. A proton singlet was observed at δ 7.2 attributable to H-3. A methylenedioxy group attached to the planar of oxoaporphine ring system gave a singlet of two protons at δ 6.32. Moreover, two sets of multiplets at δ 7.56 –7.80 and δ 8.55 –8.63 corresponding to four protons suggested that ring D was not substituted. The latter pair was

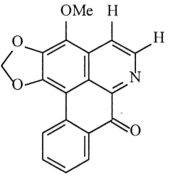
assigned to H-8 and H-11 because they are usually found more downfield than the other aromatic protons. This is due to the fact that proton on the carbon next to a carbonyl group and other electronegative atom is slightly deshielded and thus normally absorbs further downfield.

The spectral data suggested liriodenine as the plausible structure for compound PR1. This was verified by direct comparison with an authentic sample isolated earlier and also with the literature reviews [53,54,55,56,57,58,59,60,61,62]. Other names given to this compound includes oxoushinsunine, micheline-B and spermatheridine.



Scheme 10: The mass fragmentation patterns for PR1

3.1.2 Structural Elucidation of Compound Labeled PR2



This compound was crystallized from chloroform as fine yellow needles with melting point at 280°-282°C. A deep red coloration produced in acid medium suggested that PR2 to be an oxoaporphine.

The UV spectrum contained maxima at 247, 281, 312, 383 and 440 nm indicated that it possessed a 7-oxodibenzo (de,g) quinoline skeleton and thus suggested the existence of a highly unsaturated chromophore.

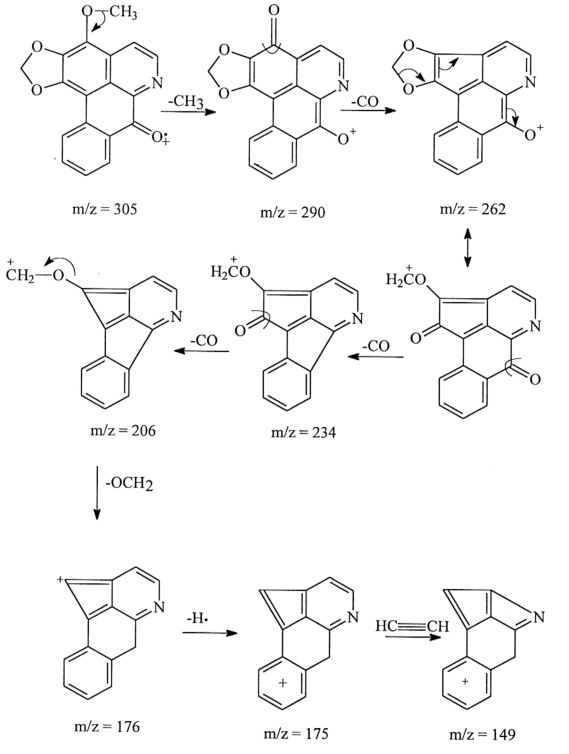
The IR spectrum of the free base showed strong absorption maximum at 1657 cm^{-1} attributed typically to a highly conjugated ketone function. Other significant peaks that help to further characterize this compound is the presence of a strong band similar to liriodenine at 750 cm^{-1}. This is due to C-H out of plane deformation of four adjacent aromatic protons in ring D. However a medium band at 957 cm^{-1} in the spectrum of liriodenine (C-H out-of-plane deformation of a single aromatic proton) was missing from that of atherospermidine. This indicated that ring A of this compound was fully substituted.

The mass spectrum revealed a strong molecular ion peak at m/z 305, thus giving the possibility of the molecular formula $C_{18}H_{11}O_4N$. The cleavage of a

methyl radical from the aromatic methoxyl substituents is exhibited by M-15 peak at m/z 290. Successive elimination of several carbon monoxide followed by CH_2O consequently revealed peaks at m/z 262, 234, 206 and 176. Subsequently, the peak at m/z 149 could be due to the loss of HC≡CH unit after rearrangement involving proton elimination. All the above descriptions of the fragmentation pathway are shown clearly in Scheme 11.

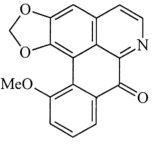
The ^{1}H-NMR spectrum of this alkaloid is almost similar to that of liriodenine, which shows a resonance of intensity three protons at δ 4.13 attributable to methoxyl group. Additional proof is by comparing these data with that for liriodenine where a proton singlet at δ 7.2 analogous to that found in liriodenine cannot be observed in the spectrum of PR2 and thus suggested that C-3 is substituted. Another singlet at δ 6.37 suggested the presence of a methylenedioxy group at C-1, 2 attached to a planar skeleton and thus confirming the isoquinoline backbone. The signals for C-5 and C-4 protons appeared as doublets ($J_{4,5}$ = 6Hz) at δ 9.04 and δ 8.18 respectively. Analyses of the signals for the remaining free protons showed them to constitute four adjacent aromatic protons resonated at δ 8.93, δ 8.53, δ 7.72 and δ 7.50 where H-11 is the most downfield due to the deshielding effect of the facing ring A.

One is justified at this stage of the discussion to conclude that alkaloid PR2 is atherospermidine or psilopine. Comparison of the empirical data with the literature values of known compound further supports this [25,53,56,59,61].



Scheme 11: The mass fragmentation patterns for PR2

3.1.3 Structural Elucidation of Compound Labeled PR3



Compound PR3 was obtained as orange-yellow needles from chloroform with melting point of 241-242°C (decomposed). The compound's high melting point together with cherry red coloration upon treatment with dilute mineral acid and failure to show N-H absorption in the infrared suggested that the highly conjugated ketone was a member of the oxoaporphine series.

Further evidence was revealed by its UV spectrum with absorption band at 211, 250, 276, 320 and 423 nm. Its UV spectrum is consonant with substitution at the C-11 position, since it lacks the strong absorption near 280 nm characteristic of 1,2,9,10- substituted aporphine. One can justify that all the three compounds discussed so far are oxoaporphine since all of them have absorption band greater than 300 nm in UV spectrum, a distinct characteristic of a carbonyl group at C-7.

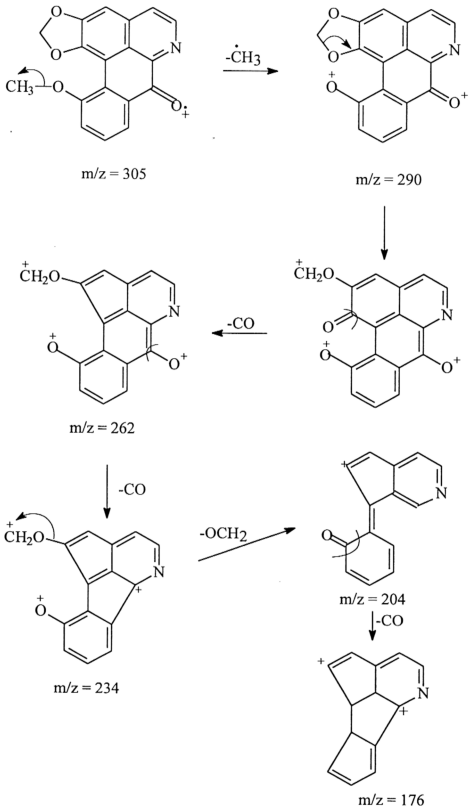
Moreover, the IR spectrum showed a conjugated ketone peak at 1724 cm^{-1}. Distinct bands, which occurred at 3021 and 2929 cm^{-1} may be assigned to the aromatic C-H stretching. The strong bands located at 1602 and 1457 cm^{-1} might be attributable to the C=C stretching of the aromatic ring. The peaks at 1244 and 1186 cm^{-1} could be assigned to the asymmetrical and symmetrical C-O-C

stretching. Similar to the previous compound, a medium peak at 975 cm^{-1} corresponds to the C-H out of plane deformation of a single aromatic proton.

The mass spectrum displayed a molecular ion peak at m/z 305, giving a possible molecular formula of $C_{18}H_{11}NO_4$. As depicted in Scheme 12, other significant fragmentations include m/z 290, 262, 234, 206, 204, 176. The first step of the scheme involved the elimination of a methyl group from the methoxyl substituent, thus suggesting that ring D is monosubstituted. Subsequent loss of carbonyl and CH_2O gave rise to the remaining peaks.

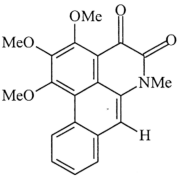
The identification of PR3 is largely based upon the ^{1}H-NMR spectrum. In this compound, the methoxyl group can be seen to resonate at δ 3.98 as a singlet with intensity of three protons while 1,2-methylenedioxy signal appeared at δ 6.28 as a two proton singlet. The spectrum also exhibited six aromatic proton signals in the δ 7.06-8.78 region. A careful analysis of this region pointed out two highly deshielded protons centered at δ 8.78 and δ 8.27. The former represented proton at C-5 (1H, d, J = 5Hz) which was highly deshielded by the neighbouring nitrogen heteroatom. Meanwhile, the latter was attributable to proton attached to C-8 (1H, d, J = 8Hz). Other aromatic signals include δ 7.64 corresponding to C-4 proton located adjacent to H-5 in ring-B since they have same coupling constant. In addition, a triplet and doublet centered at δ 7.60 and δ 7.06 with coupling constant J = 8.5 Hz were assigned to H-9 and H-10 respectively. The singlet at δ 7.12 is reminiscent of the singlet corresponding to H-3 in ring-A and thus implying that C-3 in this alkaloid is also unsubstituted.

Finally, comparison of the empirical data and the literature values of a known compound led to the conclusion that alkaloid PR3 was oxoputerine or also known as oxo-o-methylpukateine [55,63,64,65,66].



Scheme 12: The mass fragmentation patterns for PR3

3.1.4 Structural Elucidation of Compound Labeled PR4



This compound was isolated as intense and bright yellow needles crystallized from dichloromethane with high melting point at 198-201°C. Its oxoaporphinic nature is revealed by deep red colouration it produced in acid medium. Its UV spectrum showed absorption bands at λ_{max} 212, 242, 275, 306, 316 and 416 nm indicating a highly conjugated unsaturated oxoaporphine system. On acidification, the spectrum is shifted to a longer wavelength.

The IR spectrum of compound PR4 revealed peaks at 3014 and 2935 cm^{-1}, which corresponded to aromatic C-H stretching bands, and alkyl C-H bands, respectively. Methoxy moiety absorbs at 2857 cm^{-1}. A very strong and broad band located at 1660 cm^{-1} region could be ascribed to the stretching vibrations of the two-carbonyl functional group in ring-B.

The mass spectrum of compound PR4 exhibited a molecular ion peak at m/z 351, which also serves as a base peak, thus suggesting the molecular formula of $C_{20}H_{17}NO_5$. Other peak, which was also observed at m/z 336, corresponded to the loss of methyl radicals next to the nitrogen atom. Such fragmentations prove the existence of N-methyl function in the compound. In addition, the compound

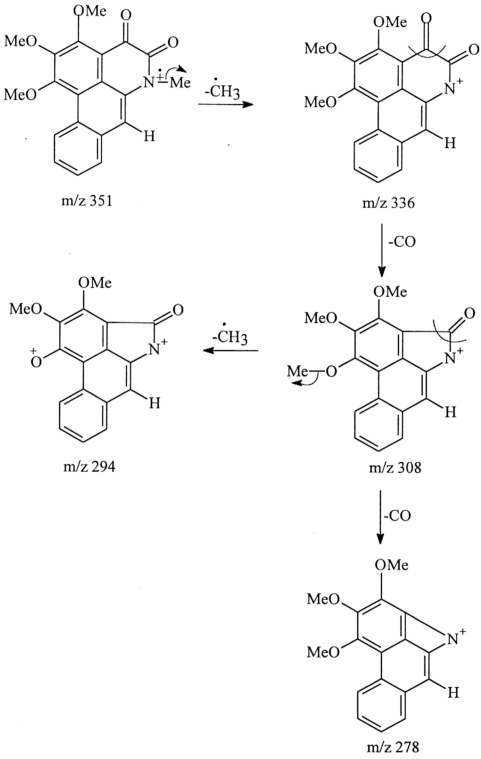
eliminates the ketone group, giving rise to a peak at m/z 308. Successive elimination of a methyl from one of the methoxyl substituents consequently revealed a peak at m/z 293. Finally, the cleavage of a carbonyl group produced a peak at m/z 278. The proposed fragmentation patterns of compound PR4 are depicted in Scheme 13.

The ^{1}H-NMR spectrum of compound PR4, as depicted in Figure 4 displayed three methoxy peaks that are very close to each other at δ 4.18, δ 4.14 and δ 4.09. These values are quite high compared to the normal signals for methoxyl groups because they are adjacent to one another. Meanwhile, an N-Me group resonates at lower field, δ 3.86 compared to normal aromatic methyl group. Unsubstituted D-ring of aporphine is evidenced from the presence of four aromatic protons found between δ 7.60 to δ 9.50 or more specifically at δ 7.66 (2H, m, H-9, H-10), δ 7.91 (1H, m, H-8) and lastly δ 9.50 (1H, m, H-11). It should be noted, moreover, that the small region between δ 7.66 and δ 7.63 exhibited two peaks where the more upfield shows a resonance of intensity two protons and appeared as a multiplet whereas the other one is a singlet and corresponds to one proton. In addition, one aromatic singlet at δ7 .61 could be due to a proton at H-7.

^{13}C-NMR spectrum displayed characteristic peaks for three methoxyl groups at δ 61.1,δ 61.6 and δ 62.1 located at C-3, C-1 and C-2 respectively. The peak at δ 30.8 is attributable to N-CH_3. In addition, carbon's signal at δ 114.9 refers to C-7 carbon. Finally, the presence of two carbonyl groups can be

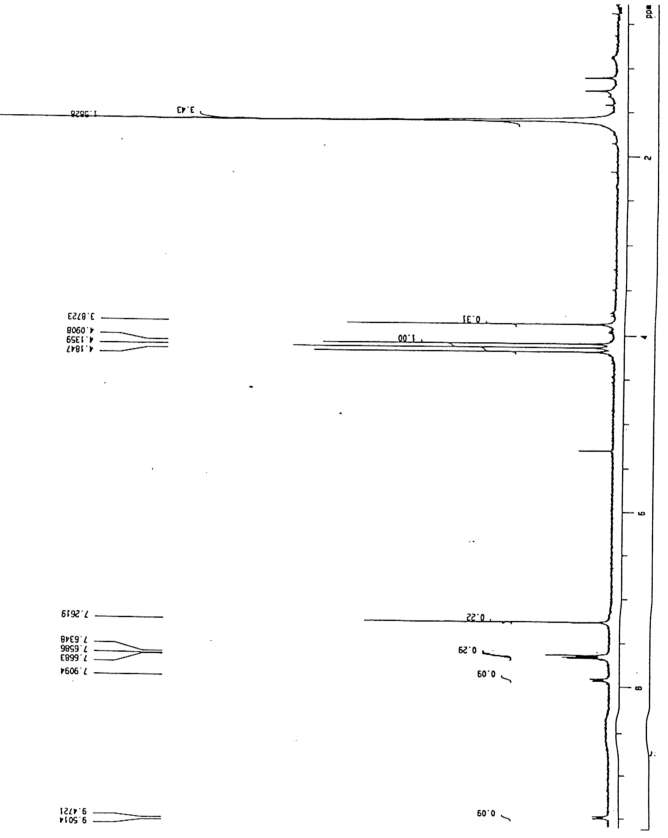
confirmed from the carbon's signal appearing at δ 174.8 and δ 160.0 in the ^{13}C-NMR spectrum.

Consequently, comparison of the empirical data with the literature values of a known compound brought to the conclusion that compound PR4 was 3-methoxycepharadione-B or also known as 1,2,3-trimethoxy-4,5-dioxo-6a,7-dehydroaporphine[67,68,69,70].

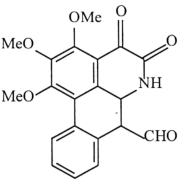


Scheme 13: The mass fragmentation patterns for PR4

Figure 4: ^{1}H-NMR Spectrum of Compound PR4



3.1.5 Structural Elucidation of Compound Labeled PR5



Compound PR5 was obtained as bright yellow needles crystallized from dichloromethane and proved to be oxoaporphine. This was apparent when it turns red upon addition with trifluoroacetic acid besides the evidence from UV and IR spectrum. Moreover, it has very low solubility and was present only in very small amount. Practically, this alkaloid was obtained from HPLC and even slightly more polar than compound PR4.

The UV-Vis maxima were observed at 212.0, 241.2, 273.3, 306, 317.6 and 416 nm, indicating a highly conjugated oxoaporphine system. On acidification, the maxima were shifted to longer wavelength. The shape of the spectrum resembles the former compound implying that the substitution pattern and also the substituents are almost the same.

However, there is a slight difference in the IR spectrum of these two compounds. A small peak could be found at about 3400 cm^{-1}, which proved the existence of the N-H functional group. Other significant peak include a conjugated ketone peak at 1678.15 cm^{-1} due to the stretching vibrations of the two conjugated C=O bonds. Besides that, several peaks typical to aromatic

compounds are as follows. Bands between 3014 cm^{-1} and 2934 cm^{-1} correspond to the stretching vibrations of the phenyl C-H and alkyl C-H bonds respectively. Those peaks at 1509 and 1462 cm^{-1} could be assigned to C=C bond stretching within the ring. Furthermore, the peak at 1230 cm^{-1} could be due to the in plane bending of the aromatic ring.

Comparison of the ^{1}H-NMR spectral data for compound PR4 with those for compound PR5 as in Figure 5 revealed close correspondence between signals for A/B/C/D ring segments of a dehydroaporphine in combination with the dioxoaporphine. However, instead of the signal for N-Me recorded in the latter compound, a proton for aldehyde group was observed at δ 10.24. This was further supported by the literature[67], where the synthesized compound, 7-formyldehydronuciferine shows peak for aldehyde group positioned at C-7 at δ 10.16. Since its NMR spectrum shows the absence of an N-Me signal and the number of protons attached to sp^2 carbon have been reduced to four, it is postulated that the CHO is substituted at C-7 proton while the N-H group replaced the N-Me. Those four aromatic proton peaks are positioned at δ 7.6, δ 7.8 and δ 9.4 where the former correspond to H-9 and H-10 whereas the most upfield signal is for proton located at C-11. The further upfield signal for methoxyl group appears at δ 4.21, δ 4.16 and δ 4.10 belonging to C-1, C-2 and C-3 respectively. This could be explained by the steric factor since they are adjacent to one another.

The mass spectrum of compound PR5 is quite distinct from compound PR4 although most of the fragmentations involved resemble to each other. It

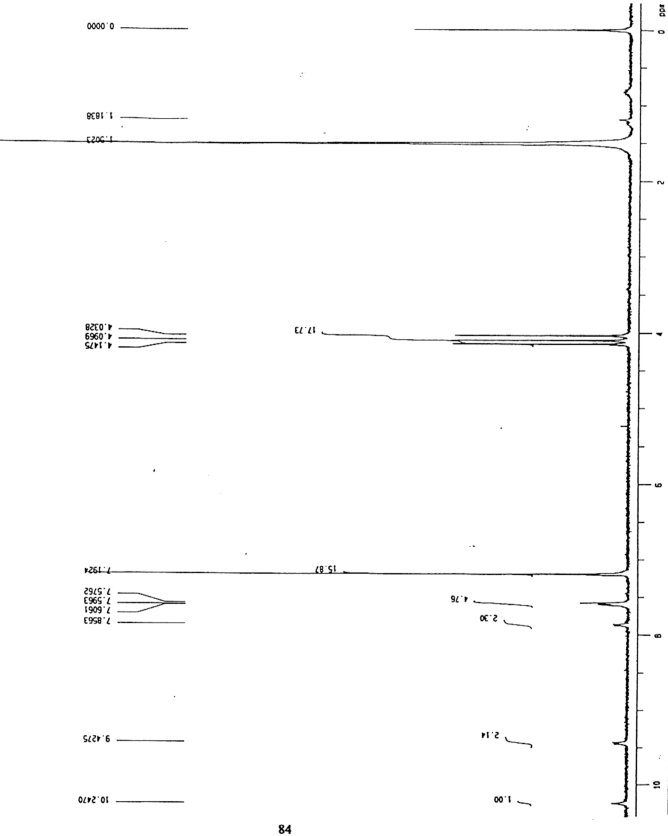
shows a molecular ion peak at m/z 362, matching to molecular formula $C_{20}H_{12}NO_6$.

^{13}C-NMR further supported the discussions made on the preceeding spectrums. The spectrum displayed typical characteristic peaks for three methoxy groups at δ 62.10, δ 61.63 and δ 62.10. Nevertheless, carbon's signal at δ 114.9 corresponding to C-7 was absent. Instead, a more downfield peak was observed at δ 199.81, accounting for the C=O of the aldehyde functional group.

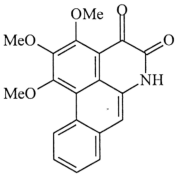
The COSY (H-H correlation spectroscopy) spectrum provided the proton-proton connectivity network. This spectrum further strengthened the discussions made on the ^{1}H-NMR of this compound, which prove the correlation between protons in ring-D.

Based on the analysed data, it was proposed that it is a new compound and has been named as rugosanine[67,71,72].

Figure 5: ^{1}H-NMR Spectrum of Compound PR5



3.1.6 Structural Elucidation of Compound Labeled PR6



Compound PR6 was afforded as red amorphous material decomposed at 280°C. The UV spectrum exhibits maxima at 213, 229, 253, 270 and 488 nm, indicating a highly conjugated system in comparison with that of dehydroaporphine. An absorption maximum or shoulder between 252 and 265 nm is diagnostic of a dehydroaporphine.

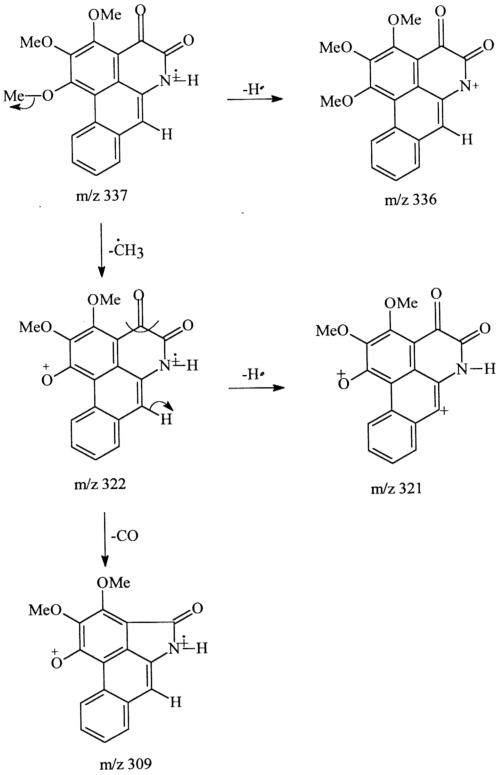
Moreover, its IR spectrum showed a conjugated ketone or six member ring lactam at 1724, 1649 and 1463 cm^{-1} in conjunction with the bands at 2997 and 2938 cm^{-1}, accounting for an aromatic C-H stretch. The presence of N-H group is evidenced from the peak located at 3436 cm^{-1}. The appearance of a medium-strength band 750 cm^{-1} is ascribable to the out of plane deformation of the four adjacent aromatic proton bands in ring–D.

Its mass spectrum established its molecular formula as $C_{19}H_{15}NO_5$ and thus implying that it is highly unsaturated. Other significant fragmentation revealed by the mass spectrum were m/z 336, 322, 321, 309 as illustrated in Scheme 14. The peak at m/z 322 is consistent with the loss of methyl unit from one of the aromatic methoxyl substituents. The fragmentation then continues with

the elimination of a carbonyl group at m/z 309. Successive losses of protons gave rise to the remaining peaks.

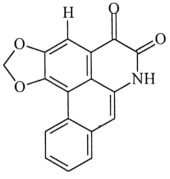
The ^{1}H-NMR spectrum showed the presence of three methoxyl groups and the rest of the spectrum consisted of five aromatic proton signals. The three distinct methoxyl peaks appeared as three singlets with intensity of three protons each at δ 4.05, δ 4.07 and δ 4.11 and most probably positioned at C-1, C-2 and C-3 respectively. Among the aromatic protons, the signal for H-11 is shifted to a lower field, which is at δ 9.07. This circumstance is typical since C-1 is substituted with a methoxyl group. Other signals for aromatic protons in ring-D includes δ 8.46 (1H, d, H-8), δ 7.76 (1H, t, H-10) and δ 7.55 (1H, t, H-9) with coupling constant J = 9 Hz. A singlet at δ 7.52, which belongs to the proton positioned at C-7, is found unusually low shielded. This fact suggested that the B-ring of the alkaloid be highly strained as expected for a 4,5-dehydroaporphine.

On the basis of the melting point and spectral behaviors as well as biogenetic considerations, this alkaloid was presumed to be ouregidione or 1,2,3-trimethoxy-4,5-dioxo-6a,7-dehydroaporphine. Moreover, the spectral data is consistent with the literature values [33,67,73,74,75].



Scheme 14: The mass fragmentation patterns for PR6

3.1.7 Structural Elucidation of Compound Labeled PR7

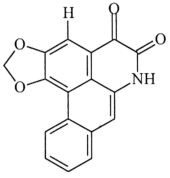


Norcepharadione-A was obtained as yellow amorphous solid. Again it also showed red coloration in acid medium suggested that it belonged to oxoaporphine skeleton.

The UV spectrum of this compound showed a characteristic of an oxoaporphine chromophore with bands at 221, 250, 264sh, 330, 360, and 470 nm. The IR spectrum, which showed two distinguishable carbonyl absorptions at 1660 and 1615 cm^{-1} further, verified this. The existence of a medium-strength band at 740 cm^{-1} would probably an indicative that ring-D was unsubstituted.

The molecular ion peak of this compound could be observed at m/z 291 in accordance with the molecular formula $C_{17}H_9O_4N$. Other fragmentations underwent by compound PR8 are featured in Scheme 15. The location of the peaks in the spectrum is 291, 290,289, 264, 263, 232, 206, 203 and 178. One of the routes is through the elimination of a proton adjacent to the nitrogen atom followed by the proton at C-7. This is evidenced from the peak at m/z 290 and m/z 289. Other possible route was the successive cleavage of a carbonyl, methyl and also ethylene unit from the aporphine skeleton as displayed by the peaks at m/z 263, 232, and 206 respectively.

3.1.7 Structural Elucidation of Compound Labeled PR7



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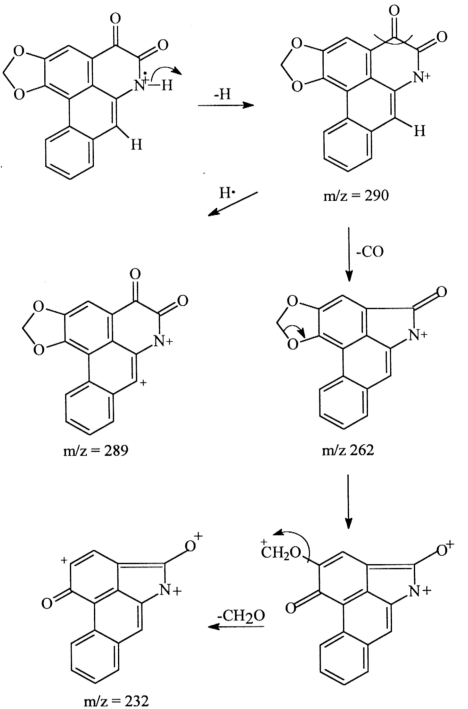
The UV spectrum of this compound showed a characteristic of an oxoaporphine chromophore with bands at 221, 250, 264sh, 330, 360, and 470 nm. The IR spectrum, which showed two distinguishable carbonyl absorptions at 1660 and 1615 cm^{-1} further, verified this. The existence of a medium-strength band at 740 cm^{-1} would probably an indicative that ring-D was unsubstituted.

The molecular ion peak of this compound could be observed at m/z 291 in accordance with the molecular formula $C_{17}H_9O_4N$. Other fragmentations underwent by compound PR8 are featured in Scheme 15. The location of the peaks in the spectrum is 291, 290,289, 264, 263, 232, 206, 203 and 178. One of the routes is through the elimination of a proton adjacent to the nitrogen atom followed by the proton at C-7. This is evidenced from the peak at m/z 290 and m/z 289. Other possible route was the successive cleavage of a carbonyl, methyl and also ethylene unit from the aporphine skeleton as displayed by the peaks at m/z 263, 232, and 206 respectively.

The ^{1}H-NMR spectrum of compound PR7 exhibits a distinct signal for methylenedioxy group at δ 6.64. Besides that, two singlets of uncoupling aromatic proton at δ 7.71 and δ 7.19. The one with further downfield shift was assigned to H-3, while the latter was ascribable to a proton at C-7. Meanwhile, four coupling aromatic protons in ring-D appeared as a multiplet at δ 9.43, δ 7.61, δ 7.57 and δ 7.90 corresponding to H-11, H-10, H-9 and H-8 respectively. The lowest field absorption at δ 11.41 in the spectrum could be due to the N-H group.

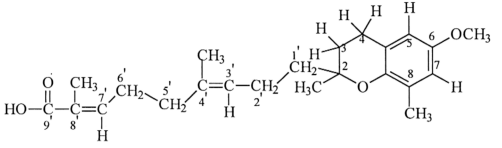
In view of the fact that the spectral data were similar to those of Norcepharadione- A, it was reasonable to conclude that this compound was Norcepharadione-A [68,70,76,77,78].



Scheme 15: The mass fragmentation patterns for PR7

3.2 Petroleum-ether Extract of *Pseuduvaria rugosa*

3.2.1 Structural Elucidation of Compound Labeled PR8



This compound was obtained as viscous brownish-orange oil. Although, it gave positive test using Dragendorff's reagent, this compound was not an alkaloid. This was based on the spectroscopic evidence. It appeared to be a new compound and there is still no report on the isolation of such compound from other *Pseuduvaria* species. Furthermore, it is the major component of the plant studied and could be obtain from pet- ether as well as dichloromethane extract.

The compound is classed under 1-benzopyran or more specifically dihydro-1-benzopyran or chroman. Historically, chroman was first prepared in 1905. However, little interest was shown in the compound until studies on tocopherols (vitamin E) began to indicate that they were derivatives of chroman. The tocopherols form a group of 6-chromanols. Chromans could contain more than one functional group. Nearly all the naturally occurring chromans have long isoprenoid chains, attached particularly at C-2, and most are members of the tocol series. Typically, this class of compound which contain a carboxyl group either at C-2 or in a chain attached to C-2 have been studied as antioxidants. The carboxylic acid has a synergystic effect on the antioxidant activities of ascorbic

acid and ascorbyl palmitate. However, very few of chroman carboxylic acids were reported to exist in nature[79, 80, 81].

It's UV spectrum revealed a fairly intense absorption band at 210 nm together with three other maxima at 240, 270 and 300 nm, thus, suggesting a highly conjugated system. These values were comparable with those of other chroman carboxylic acids as reported in the literature[80]. This is because aromatic rings do contain a conjugated π electron system. Similarly, unsaturated acids display a strong K-band characteristic of the conjugated system.

In the IR spectrum of compound PR8, an absorption characteristic of a conjugated carbonyl function at 1689 cm^{-1} was observed. Meanwhile, the O-H bond of the carboxyl group gave rise to a very broad absorption at about 3500 cm^{-1}. Other peaks observed were at 3049 and 2932 cm^{-1}, which corresponded to the C-H stretching of the alkyl and alkene group. Moreover, the bands between 1631 and 1476 cm^{-1} could be assign to the C=C stretching of the benzene ring and alkene. An intense peak at 1244 cm^{-1} might be attributable to the phenolic C-O absorption of the chroman group. In addition, multiple bands observed in the range 1187 to 1061 cm^{-1} could be due to the asymmetric and symmetric C-O-C stretching of ethers.

The mass spectrum (Figure 6) revealed a molecular ion peak at m/z 372, suggesting a possible molecular formula of $C_{23}H_{32}O_4$. Generally, the mass spectrum of compound PR8 is characteristic of a chroman which is substituted with straight chain carboxylic acids. The prominent peak that could be observed from the spectrum was at m/z 191. As shown by Scheme 16, the cleavage

occurred at the highly substituted carbon atom, C-2 of the chroman ring. The peaks of the straight-chain carboxylic acids were quite discernible. Weak peaks of M-17 and M-45 represented cleavage of bonds next to the carbonyl group. Besides that, the spectrum of the long-chain acid resemble the series of typical hydrocarbon clusters at interval of 14 mass units which could be seen as small peaks at m/z 311, 297, 283, 269, 259, 250, 241, 230, 218 and 206.

However, most of the distinct peaks displayed by the spectrum were due to the fragmentation underwent by the chroman ring with methoxy and methyl group attached to the benzene ring. Significant fragmentation exhibited by the mass spectra were m/z 191, 163, 151 (100%, base peak), 135, 121, 107, 91, 77, 67, and 55. The proposed fragmentation well illustrated by Scheme 16 suggested that chroman underwent two types of fission. The first path involved the loss of C-2, which occurred with hydrogen transfer to give the base peak at m/z 151 fragment. After subsequent losses of methyl and methoxyl group, a tropylium ion was preferably produced at m/z 107, partly by analogy with known ring expansions of benzylium ion. Consequently, a characteristic cluster of ions due to the α cleavage and hydrogen migration in monoalkylbenzene appeared at m/z 77 ($C_6H_5^+$) and 79 ($C_6H_7^+$). Finally, the elimination of a neutral acetylene molecule (HC≡CH) from the tropylium ion resulted in a peak at m/z 67. Alternatively, for the second path, chroman passed through what was probably the same ion as that observed in the spectrum of dihydrobenzofuran to give fragment ions at m/z 191 followed by 163. This corresponded to the chromene ion and the usual ether fission. Further breakdown of this occurred at m/z 91 due to the $C_7H_7^+$ ion [71, 82].

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Figure 7 illustrates the integrated 500-MHz ^{1}H-NMR spectrum of compound PR8. From left to right the integration of the ^{1}H specrum shows 1, 1, 1, 1, 3, 2, 2, 3, 2, 2, 3, 2, 3, 2, 3 protons for a total of 31 protons. Obviously, the four low–field absorptions as exhibited in the figure represent the aromatic and alkene methine protons while the remaining were high-field protons comprising of a methoxyl group, four methyl groups and six methylene protons. The two doublets at δ 6.56 and δ 6.44 corresponded to H-7 and H-5 respectively, that was meta coupled to each other. This was apparent from their coupling constant, J = 2.8 Hz. The signals for the two-aryl protons absorb further downfield because they were deshielded by the diamagnetic anisotropy of the π-electrons. The broad triplets at δ 6.06 and δ 5.16 could be assigned to olefinic protons of H-7' and H-3' in the aliphatic chain. The former was highly deshielded by the neighbouring carbonyl group. Besides that, the coupling constant of this proton is 7.2 Hz which matched to the adjacent H-6' (δ 2.62, q, J = 7.2 Hz). Meanwhile, the latter has J value of 7.9 Hz, thus, indicating that it was coupled to H-2' (δ 2.12, t, 2H, J=7.9 Hz). Other methylene signal appeared at δ 2.07 (2H, t, J=7.2 Hz). From the splitting and the J value of the potons discussed previously, it could be deduced that coupling of H-5' and H-7' with the adjacent methylene , H-6' resulted in quartet (J = 7.9 Hz). Two singlet of allylic methyl group in the chain resonated at δ 1.90 (8'-CH_3) and δ 1.64 (4'-CH_3).

The aromatic region of the ^{1}H-NMR spectrum was typical of that expected for a chroman. The 3- and 4-methylenes of the chroman ring showed as triplets (J = 6.8 Hz), probably due to equilibration in the pucker of the ring system, resulting

in an A_2X_2 system. The methylene next to the benzene ring (H-4) was most affected by the deshielding of the circulating π-electrons and can be found at lower field, δ 2.73. Meanwhile, the methylene protons of H-3 were not equivalent due to the effect of chiral center where the carbon chiral was at C-2 of the chroman ring. The nonequivalent protons of the H_a and H_b absorbed, individually, at ~δ 1.75 and ~δ 1.80. The chemical shift of other methylene protons, C-1' were also nonequivalent due to the same effect of the chiral center in the molecule. The H_a of C-1' appeared at δ 1.55 whereas the H_b was detectable at δ 1.65. They couple with each other, and each may have a different coupling constant to a vicinal proton resulted in a multiplet. The methyl attached to the aromatic ring of chroman (8-CH_3) absorbed at a more downfield position, which was at δ 2.15 compared to a methyl group substituted at C-2 position that resonated at δ 1.26. Finally, a strong peak at δ 3.73 corresponding to three protons would be due to the methoxy directly attached to the aromatic ring [40, 79, 81, 83, 84].

The ^{13}C-NMR spectrum was exhibited in Figure 8. By assuming each peak represents a single ^{13}C, the spectrum shows 23 ^{13}C atoms. The DEPT (distortionless enhancement by polarization transfer) spectrum of compound PR8 as shown in Figure 9 enhance the existing ^{13}C spectrum. This useful technique distinguished the CH_3, CH_2, CH as well as quartenary carbon peaks. Peaks shown after the $90°_y$ pulses are attributable to CH of the ethylene and aromatic ring. Peaks shown after the 135° delay are assigned to CH (up) of the ethylene and aromatic ring, CH_3 (up) of the terminal methyl and CH_2 (down). Hence, it could

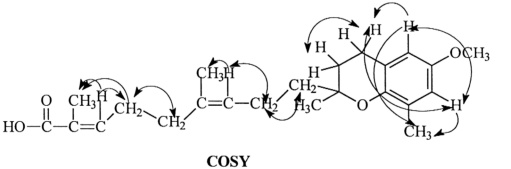
be deduced that there are seven quaternary carbons, six CH_2, four CH_3, four CH and one-methoxy signals.

The most downfield peak at δ 173.5 was attributable to the carbonyl atom of the carboxylic acids. The quartenary carbon bonded to the methoxy group resonated at δ 152.5, which was typical to aromatic carbon *ipso* to oxygenated functional group. A signal at δ 56.5 corresponded to the methoxy carbon. The two sp^2 carbon atoms of alkenes, i.e. C-7' and C-3' gave signals at δ 146.7 and δ 125.6 respectively. The former experienced a deshielding effect from the C=O due to its β position from the group. This was caused by the fact that delocalization of electrons resulted in a lower electron density at the β proton.

The quaternary carbon adjacent to the oxygen atom (C-8a) gave rise to a peak at a downfield position at δ 146.4 due to the deshielding effect caused by the neighboring oxygen atom of the chroman ring while the substituted aromatic carbon resonance for C-4a appeared at δ 121.3. Chemical shifts of the two-protonated aromatic carbon at δ 111.4 and δ 115.2 were assigned to C-5 and C-7 respectively. The downfield shifts of the substituted aromatic carbon compared to the protonated one are merely a consequence of a deshielding effect of the adjacent methyl or methoxy group. The carbon resonance of the Me group bonded to an aromatic ring (C-8) was at δ 16.6. The C-3 and C-4 methylene signals could be observed at δ 31.8 and δ 23.1 while the quaternary carbon at C-2 absorbed at about δ 75.7. The methyl group directly attached to this carbon exhibited a signal at δ 24.4. The peaks at δ 134.6 and δ 126.6 might be attributable to the quaternary carbons, C-4' and C-8', respectively. The upfield signals at δ16.1 and δ20.9

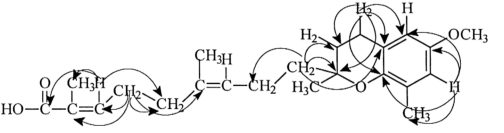
corresponded to the methyl carbon directly attached to C-4' and C-8'. The remaining peaks at δ 40.2, δ 39.4, δ 28.5 and δ 22.6 were thus assigned to the methylene carbon atom of the aliphatic chain C-1', C-5', C-6' and C-2' respectively[79, 81, 85]. The tentative assignment of the chemical shifts discussed above was well illustrated in Table 7.

COSY (H-H correlation spectroscopy) spectrum as depicted in Figure 10 provided almost all the ^{1}H-^{1}H connectivity effectively. It is one of the most useful tools of all 2-D spectra, which further strengthened the assignments, and discussions made on the ^{1}H-NMR spectra. The spectrum emphasized the meta coupling of H-5 and H-7 of the aromatic protons as well as their long range coupling to H-4 and the methyl group directly attached to the aromatic ring. Besides that, the crosspeaks of H-3 and H-4 supported their positioning in the chroman ring. In addition, the position of the protons in the aliphatic chain were deduced from the correlation between H-1' and H-2' as well as H-2' and H-3' with their long range coupling to 4'-CH_3. The coupling of the remaining protons in the chain were apparent through the off-diagonal crosspeaks H-5'/ H-6' and H-6' / H-7'. Also apparent is the long-range coupling of the H-6' and H-7' to the methyl group bonded to C-8'.



COSY

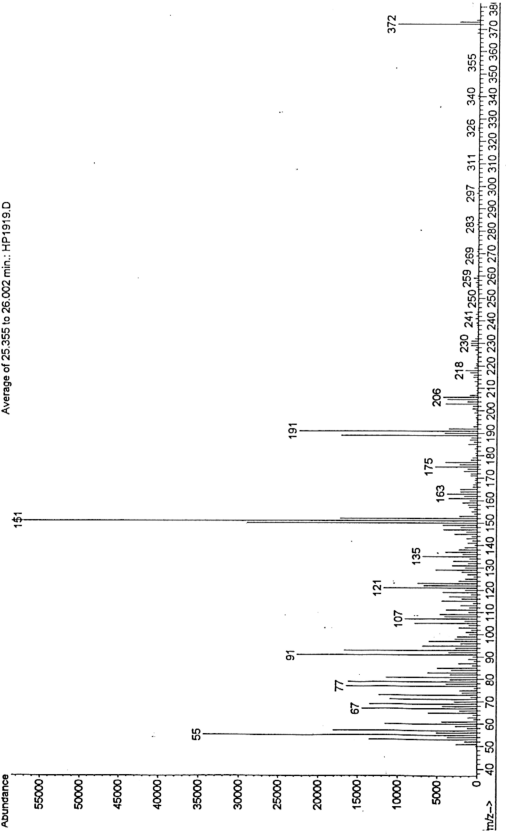
The HMQC spectrum as pictured in Figure 11, identified the specific protons directly attached to each carbon atoms except for quaternary carbon atoms. Correlating the peaks of ^{1}H spectrum with those of ^{13}C spectrum resulted in the specific assignments of all the carbons and protons as presented in Table 7. However, careful analysis of HMBC (heteronuclear multiple bond connectivity) as in Figure 12, completed the structural assignments and consequently, led to the structure for compound PR8. The HMBC experiments gave information on heteronuclear shift correlation via long-range C-H couplings (7-10 Hz) usually from ^{2}J and ^{3}J. Correlation from H-1' to C-2', C-3, C-2, C-4a and from H-6' to C-4', C-5', C-7', C-8' as well as from H-4 to C-2, C-3, C-4a, C-8a and C-5 were particularly diagnostic in determining the overall structure of 2-substituted chroman . Finally, other important correlation that supported the discussions made in the preceding spectrum includes H-7'/ C-5, C=O, 8-CH_3 and H-7 / C-6, C-5, 8-CH_3, C-8a [79].

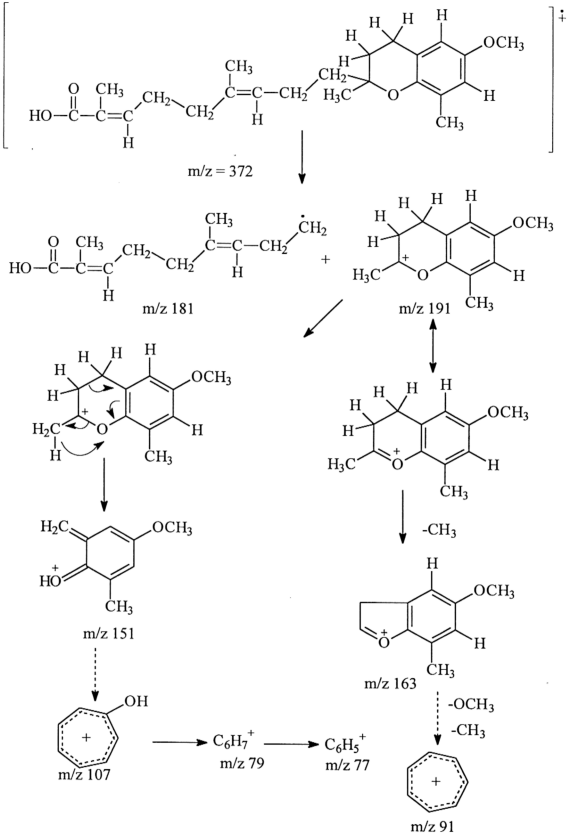


HMBC

From the observed data, it can be concluded that the compound PR8 has the structure as shown above and was named as rugosin-A.

Figure 6: Mass Spectrum of Compound PR8





Scheme 16: The mass fragmentation patterns for PR8

Table 7 : ^{13}C-NMR and ^{1}H-NMR Data for Compound PR8

Position	^{13}C-NMR (ppm)	^{1}H-NMR ppm (J Hz)
COOH	173.5	
1		
2	75.7	
3	31.8	1.80 (m, 1H, C_3-H_a) 1.75 (m, 1H, C_3-H_b)
4	23.1	2.73 q (6.8)
4a	121.3	
5	111.4	6.44 d (2.8)
6	152.5	
7	115.2	6.56 d (2.8)
8	127.6	
8a	146.4	
1'	40.2	1.55 (m,1H,$C_{1'}$-H_a) 1.65 (m,1H,$C_{1'}$-H_b)
2'	22.6	2.12 t (7.9)
3'	125.6	5.16 t (7.9)
4'	134.6	
5'	39.4	2.07 t (7.2)
6'	28.5	2.62 q (7.2)
7'	146.7	6.06 t (7.2)
8'	126.6	
2-CH_3	24.4	1.26 s
6-OCH_3	56.5	3.73 s
8-CH_3	16.6	2.15 s
4'-CH_3	16.1	1.64 s
8'-CH_3	20.9	1.90 s

Figure 7: ^{1}H-NMR Spectrum of Compound PR8

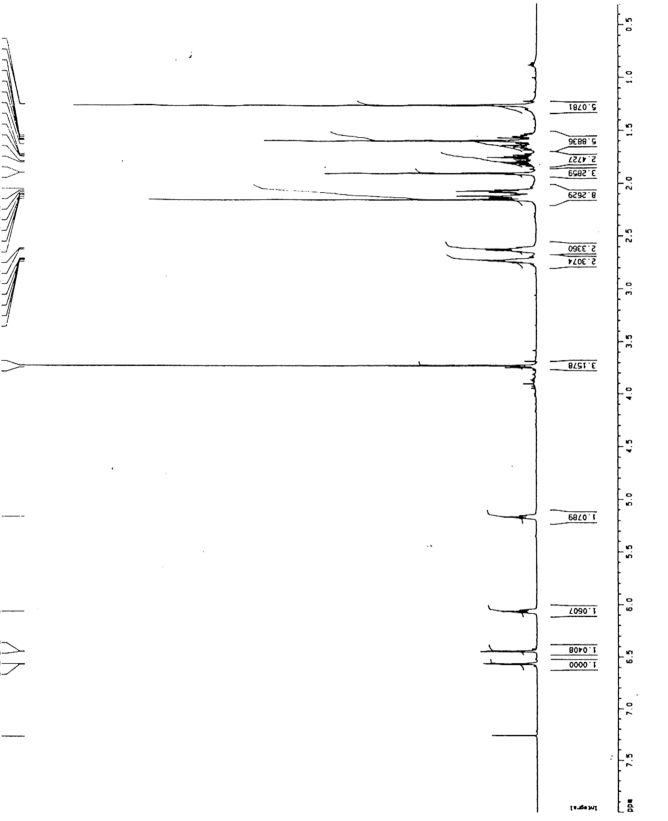


Figure 8: ^{13}C-NMR Spectrum of Compound PR8

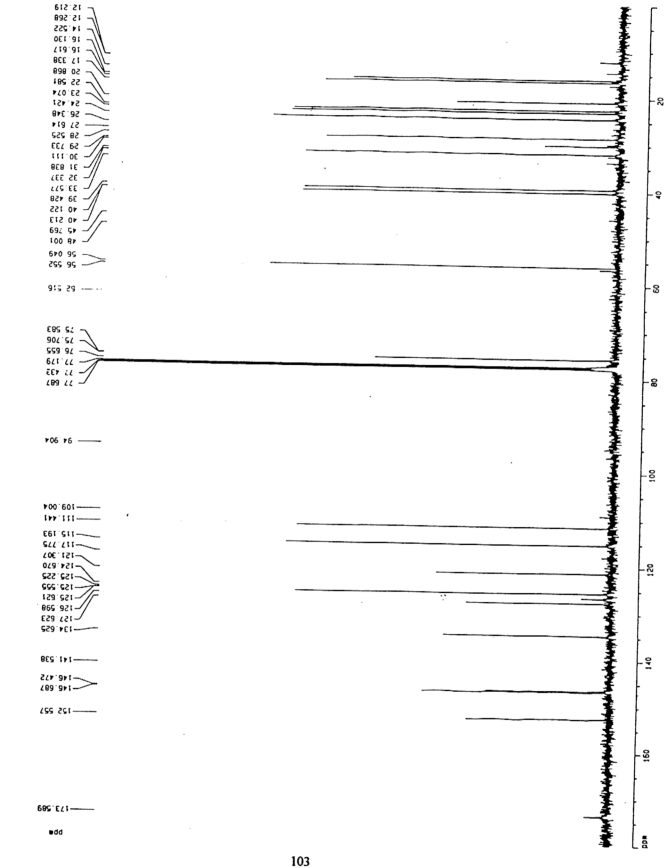


Figure 9: DEPT Spectrum of Compound PR8

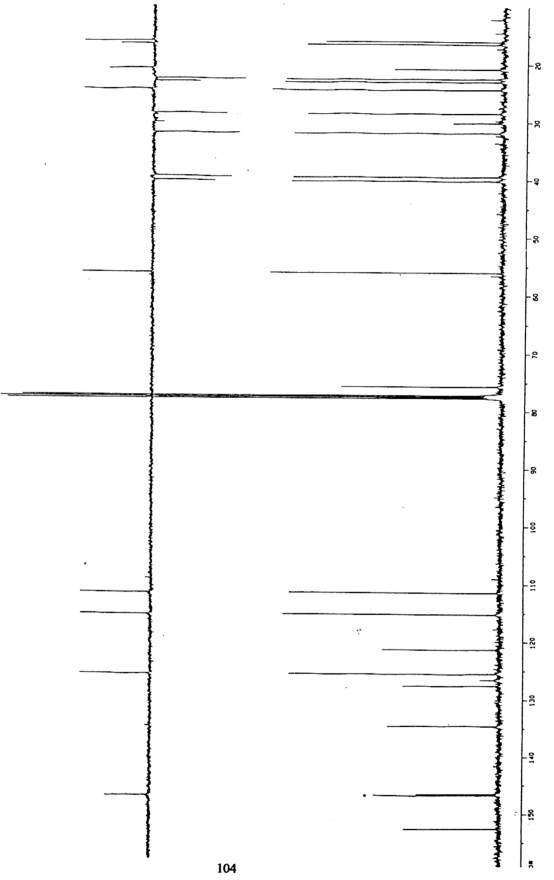


Figure 10: COSY Spectrum of Compound PR8

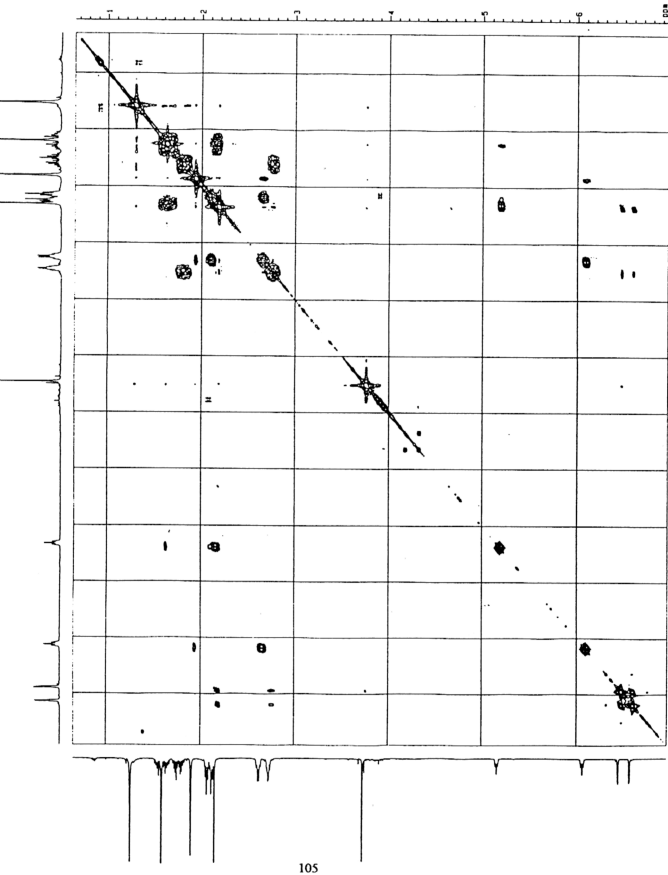


Figure 11: HMQC Spectrum of Compound PR8

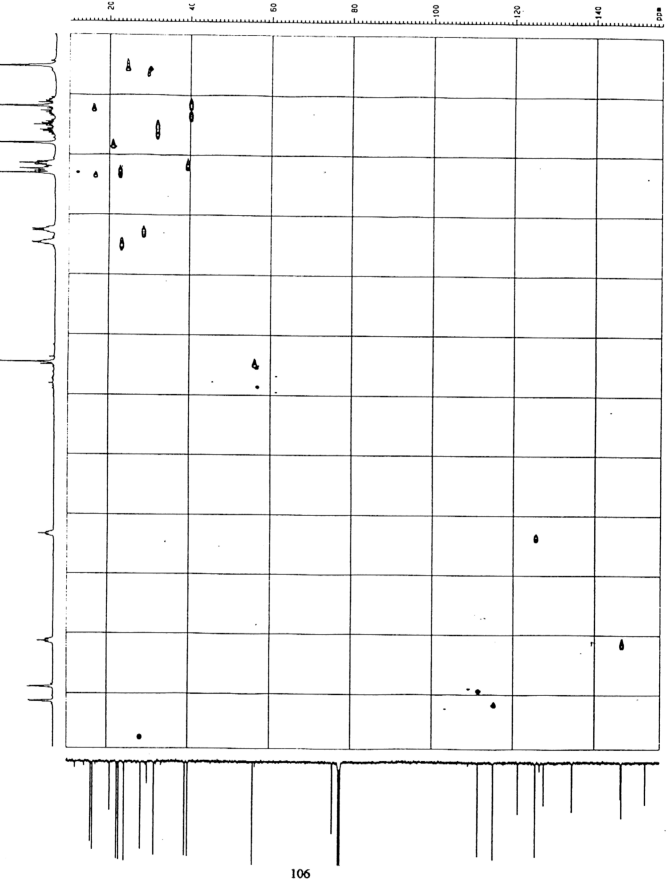
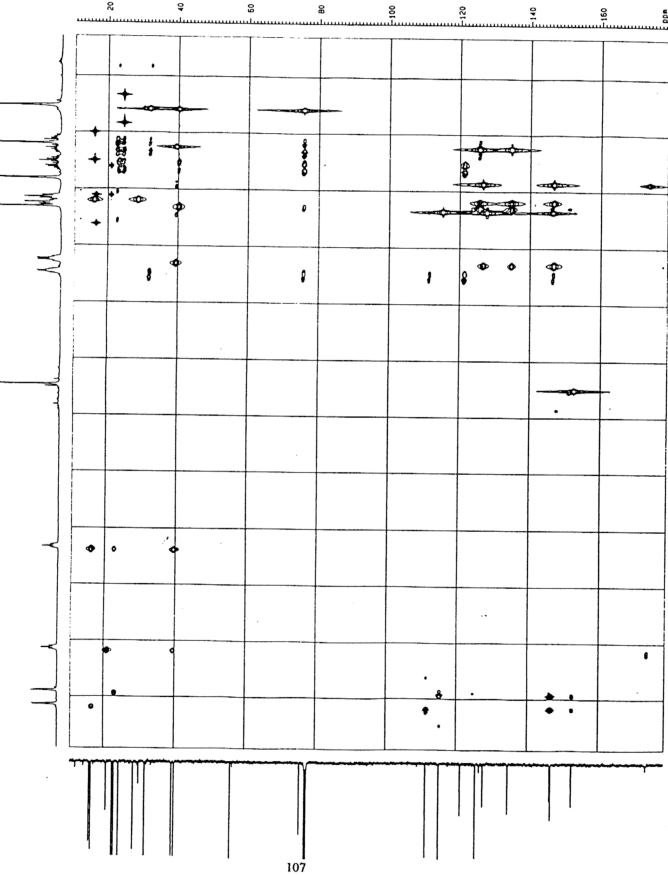
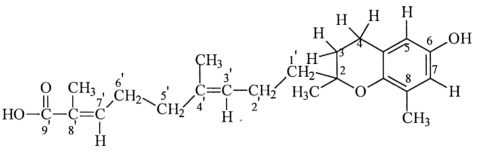


Figure 12: HMBC Spectrum of Compound PR8



3.2.2 Structural Elucidation of Compound Labeled PR9



Compound PR9 was afforded as viscous brown oil. It belongs to tocopherol or chroman-6-ol group where position 6 of the chroman ring is substituted with OH group. This compound was obtained from petroleum-ether extract together with PR8.

The UV spectrum of PR9 exhibited two maximum absorption at 215 and 298 nm, hence, indicating the presence of conjugated unsaturated aromatic system.

The most distinct peak observed in the IR spectrum for chromanols result from OH stretching vibrations at 3514 cm^{-1}. The presence of conjugated carbonyl function in the isoprenoid chain was evidenced from an intense peak at 1692 cm^{-1}. Absorption peaks between 3021 and 2931 cm^{-1} could be due to the phenyl C-H and alkyl C-H bonds, respectively. Besides that, the strong peak at 1241 cm^{-1} could be assigned to the phenolic C-O absorption whereas multiple peaks in the range from 1188 to 1091 cm^{-1} might be attributable to the C-O stretching.

The mass spectrum of compound PR9 revealed a molecular ion peak at m/z 358, corresponding to the possible molecular formula $C_{22}H_{30}O_4$.

Basically, the ^{1}H-NMR spectrum of PR9 as shown in Figure 13, resembled those of PR8. However, most of the peaks especially the highfield side were not well resolved, resulting in several overlapping peaks. Obviously, the methoxy peak at δ 3.73 was absence in PR9. Therefore, it was postulated that C-6 of the chroman ring was substituted with O-H group. Other proton signals were almost the same but shifted slightly upfield as compared to PR8.

Similarly, the ^{13}C-NMR spectrum of PR9 (Figure 14) was comparable to PR8. The missing of methoxy signal at δ 56.5 as well as the further upfield signal of the quaternary carbon directly attached to the functional group further verified that methoxy group at C-6 in PR8 was replaced by hydroxyl group in PR9. Table 8 displays the data of ^{13}C-NMR and ^{1}H-NMR of this compound.

Consequently, it was reasonable to conclude that PR9 has the structure as shown above and has been referred to as rugosin-B.

Table 8: ^{13}C-NMR and ^{1}H-NMR Data for Compound PR9

Position	^{13}C-NMR (ppm)	^{1}H-NMR ppm (J Hz)
COOH	173.4	
1		
2	75.4	
3	31.4	1.4-1.7
4	22.3	2.5-2.6 (Ha), 1.9-2.1(Hb)
4a	121.2	
5	112.6	6.28 d (2.7 Hz)
6	147.7	
7	115.6	6.37 d (2.7 Hz)
8	127.4	
8a	145.7	
1'	39.6	1.4-1.7
2'	22.2	1.9-2.1
3'	125.3	5.06 t (6.1 Hz)
4'	134.1	
5'	39.5	1.9-2.1
6'	28.0	2.57 q (7.4 Hz)
7'	146.2	5.97 t (7.1 Hz)
8'	126.7	
2-CH_3	23.9	1.21 s
6-OCH_3		
8-CH_3	15.9	2.02 s
4'-CH_3	12.2	1.53 s
8'-CH_3	20.3	1.81 s

Figure 13: ^{1}H-NMR Spectrum of Compound PR9

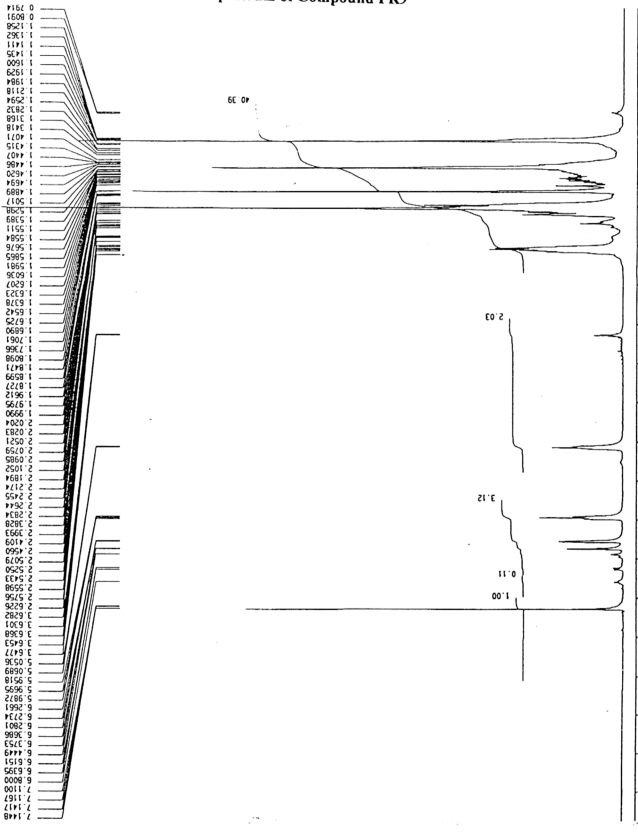
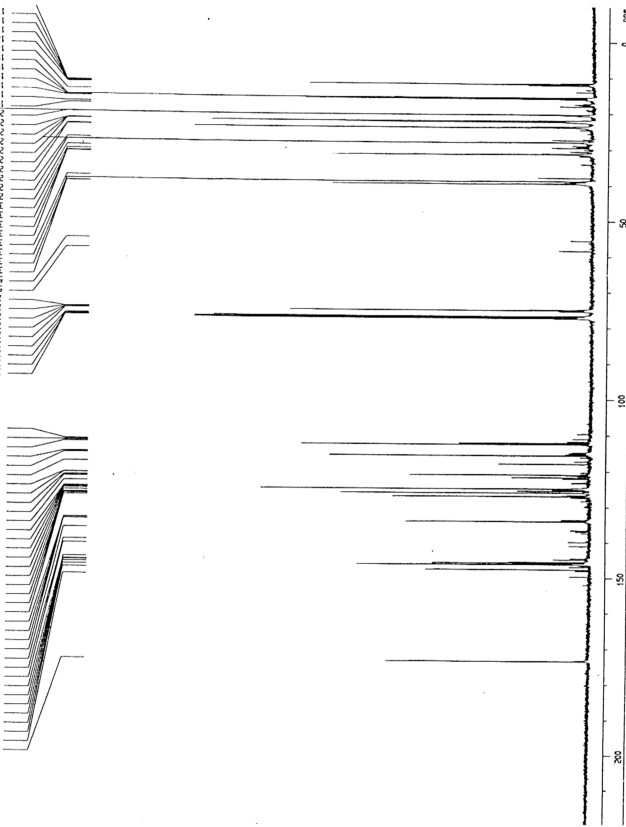


Figure 14: ^{13}C-NMR Spectrum of Compound PR9



3.3 Conclusions

The phytochemical investigation of the stem bark of the *Pseuduvaria rugosa* has led to the isolation and identification of nine interesting compounds. From that number, two non-alkaloidal compounds have been successfully isolated from the pet-ether extract and classified as chromans, while the remaining were obtained from the CH_2Cl_2 extract of the plant, mostly alkaloidal constituents. To that extent, it is gratifying to note that the latter belong to the same class of compound namely aporphine alkaloid or more specifically oxoaporphines. The following lists all the isolated compounds and the group they belong to:

- oxoaporphine: liriodenine, atherospermidine and oxoputerine.
- 4,5-dioxoaporphine: ouregidione, 3-methoxycepharadione-B, rugosanine, norcepharadione-A
- chroman: rugosin-A, rugosin-B

Only rugosanine appeared to be a new alkaloid, while the rest are known compounds. Both chroman isolated from this species are new compounds and never been isolated before either in the Annonaceae plant nor other family of plant.

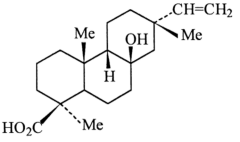
The structures of the molecule were established through spectroscopic methods such as UV, IR, MS and NMR. Where necessary, 2D- NMR such as HMBC, HMQC, COSY were also employed to assist the identification of more complicated structure. In general the UV spectrum indicated the presence of the highly unsaturated chromophore of the oxoaporphine alkaloid while the IR exhibited peak attributable to carbonyl group of the oxoaporphines besides

showing the presence of N-H and O-H substituents. However, NMR method is the most useful technique especially ^{1}H-NMR in determining the structure of the oxoaporphine. It is found that ring A is mostly substituted while ring–D of the oxoaporphine skeleton is either unsubstituted or monosubstituted.

Furthermore, studies related to the family and species as reported in the literature, make the job easier by filtering possible structure of the chemical constituents of the plant. However, there are very few previous work recorded on the *Pseuduvaria* species, which includes *Pseuduvaria grandifolia, P. dolichonema, P.macrophylla* and the recent one is *P. indochinensis*. This has been discussed in earlier chapter. Basically, they produced mostly aporphinoid alkaloids. In addition, *P. indochinensis* is reported to contain diterpenes known as ent-8α-hydroxypima-15-en-18-oic acid **36**, ent-16α,17-dihydroxyprimar-15-en-18-oic-acid **37** and ent-16α,17-dihydroxyhauran-19-oic acid **38** [86]. On the basis of this, phytochemical examination on this genus as well as on the same species should be intensified. One could still observes the difference in chemical contents despite the fact that the plant belong to the same species because of genetic, different location of the plant collected; and different part and age of the plant. In short development changes and environmental factors are among the important causes of this variation. Moreover, the study revealed the great chemical diversities of plants. Consequently, it should always be coupled with biological activity test to check for their medicinal importance.

So far, there is no claim yet over the traditional usage of this plant as a herbal cure for ailments and diseases nor has economic importance. Hopefully, the study

of the chemical constituents of this plant could potentially led to biological activity test to check for their valuable pharmacological properties and later to be developed as commercial drugs. This is based on the fact that alkaloids do manifest significant pharmacological activity. Besides that, a more sophisticated and modern techniques should be employed in the experimental procedure in order to get better and efficient results.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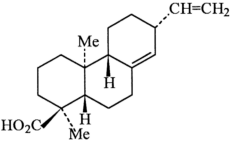
Me
H
HO2C
Me

36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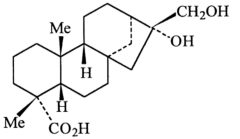
Me
H
HO2C
H
Me

37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OH
H
H
Me
CO2H

38